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Analysis of pectin content and degree of polymerization in orange juice

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A study was conducted where orange juice pectin and reducing endgroups were measured to determine the degree of polymerization of the pectic substances in freshly-squeezed orange juice. Measurement of these parameters in a freshly squeezed juice and measurement after one week of 5°C storage elucidate changes occurring during natural enzymatic clarification of the juice. The characteristics of the juice pectic substances may influence juice quality such as haze stability and viscosity (mouthfeel), and aid in determining ideal harvesting/processing dates and conditions. In addition to the documented pectinesterase activity in orange juice and its role in cloud destabilization, active polygalacturonase is indicated.

INTRODUCTION

Destabilization of orange-juice cloud has been attributed to pectinesterase activity (Versteeg, 1979); however, research also indicates differences in the clarification of fresh juices from different orange varieties which cannot be related to overall pectinesterase activity (Krop, 1974). An increase in pectinesterase (PE) activity was found with increasing maturity of the orange (Rouse & Atkins, 1953; Tahir *et al.*, 1975), and research involving other fruits implies a relationship between polygalacturonase (PG) activity and the loss of firmness associated with the ripening process and other mechanical changes occurring during growth in plant cell walls (Burns, 1991). Since nonpasteurized freshly squeezed orange juice contains active PE, it saponifies the pectin which becomes sensitive to cations and, depending upon the size of the pectin molecules, may result in insoluble pectates, thus leaving a clear supernatant (Krop, 1974).

Baker and Bruemmer (1972) treated freshly pressed orange juice with Klerzyme, containing PG and PE, and the cloud was stabilized. They attributed the stabilization to degradation of the pectic substances into soluble low molecular weight pectates which prevents the formation of high molecular weight insoluble pectates.

In studies conducted by Termote *et al.* (1977), pectic acid hydrolysates with varying degrees of polymerization were added to orange juice. Clarification occurred only when the pectate chains had blocks of 16 or more monomers with free carboxyl groups. Acid soluble pectic acid hydrolysates with an average degree of polymerization (DP) value of 12 had the highest cloud stabilizing effect.

Riov (1975) reported polygalacturonase activity in the flavedo, albedo and pulp of Shamouti and Valencia oranges; however the activities were very low and previously not detected.

In this study, our objectives were to measure the pectin and galacturonic acid reducing endgroups of orange juice, freshly squeezed, unpasteurized and stored at 5°C of various varieties in order to obtain DP values of the pectic material. In doing so, factors such as enzymatic activity and polymer size of the pectic substances can be related to clarification of the orange juice. DP has proved to be a valuable tool in the study of other fruit juice systems and may prove equally valuable as applied to orange juice.

METHODOLOGIES

Whole orange juice may be analyzed for pectin content using the *m*-hydroxydiphenyl photocolorimetric assay as described by Blumenkrantz and Asboe-Hansen (1973). However, if final sample dilutions contain more than 200 μg/ml nonuronide material, a correction factor must be applied to sample backgrounds (Kintner & Van Buren, 1982).

The degree of polymerization of the pectin can be calculated using the pectin concentration and amount of reducing endgroups based on galacturonic acid. Hexuronic acids can be sensitively quantified using the modified acidic copper acetate assay of Milner and Avigad (1967). However, chelating agents including

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citrate and EDTA pose problems in the assay. In addition, fructose for example, may contribute to the absorbance readings since it weakly reacts with the copper agent (Milner & Avigad, 1967). The above assays are sensitive, rapid, and involve photocolorimetric determination of the product. All measurements, however, can be problematic due to the large amounts of sugars and citric acid in orange juice samples. These problems can be directly avoided if the orange juice samples are precipitated with 80% ethanol, washed, dried, and resuspended to yield pectin free of non-bound carbohydrates and acids (Kertesz, 1951).

EXPERIMENTAL

Ethanol precipitations of whole juice and serum

Freshly squeezed orange juice of various varieties and packing houses was obtained through Wegman's Food Markets and processed the same day. At Wegman's, clean oranges held in the cold until used, were placed in an single-head FMC extractor. The orange juice was analyzed for %TA, pH, and °Brix and stored at 5°C. Two 200-300 ml samples were immediately homogenized using a Kinematica Polytron at speed #7 three times, each for a duration of ten seconds.

Whole juice

Each homogenized 22 g sample was transfered in duplicate into 250 ml Nalgene centrifuge tubes and 110 ml of 95% ethanol were added. The samples were centrifuged using a Sorval Superspeed model at 5°C, 5500 rpm (~5000 *g*) for 30 min. All samples were treated identically with ethanol following the methodology of Kertesz (1951). The supernatants were discarded and the pellets washed with 100 ml of 80% ethanol and centrifuged as above. The wash and centrifuge steps were again repeated. At times the pellets were not firm and the supernatant was carefully poured through a Whatman #1 qualitative filter to obtain the pellets in their entirety. The pellets were dried for 5-6 h at room temperature in a hood to rid the samples of any ethanol remaining, and finally resuspended in 20 ml of water containing 0·001% Geneticin (Sigma, antibiotic G418, disulfate salt). The suspensions were stirred for approximately 24 h at 5°C and subsequently analyzed.

Serum

From each homogenate an aliquot of the orange juice was transfered in duplicate into 250 ml Nalgene centrifuge tubes until they were three quarters full. These were then centrifuged using a Sorval Superspeed model at 5°C, 1500 rpm (~370 *g*) for 10 minutes. The supernatants were pipetted off and filtered through #1 qualitative Whatman filter paper to obtain the serum free from the centrifuged pulp. As done for the whole juice, from each serum sample, 22 g were transfered into 250 ml Nalgene centrifuge tubes and 110 ml of 95% ethanol were added. The samples were centrifuged using a Sorval Superspeed model at 5°C, 5500 rpm (~5000 *g*) for 30 min. The supernatants were discarded and the pellets washed with 100 ml of 80% ethanol and centrifuged as above. The wash and centrifuge steps were again repeated. At times the pellets were not firm and the supernatant was poured through a Whatman #1 qualitative filter to obtain the pellet material in its entirety. The pellets were dried for several hours at room temperature in a hood to rid them of any ethanol remaining, and finally resuspended in 20 ml of water containing 0·001% Geneticin. The suspensions were stirred for approximately 24 h at 5°C and subsequently analyzed.

Pectin content and degree of polymerization

Diluted samples were tested for pectin concentration using the *m*-hydroxydiphenyl assay (Kintner & Van Buren, 1982). Reducing endgroups were determined using the modified acidic copper acetate assay (Sajjaanantakul *et al.*, 1989). Centrifugation at the end of the assay prior to reading absorbances was necessary to remove precipitate. A table-top clinical centrifuge was used at full speed for 10-15 min, then tubes were read. Results are given in the units μmole/g orange juice. The degree of polymerization (DP) of a sample was calculated using moles of galacturonic acid divided by moles of uronic reducing groups, yielding an average polymer size of the pectin.

RESULTS

As seen in Table 1, the pectin content of the whole juice remained constant over a period of one week at 5°C for all varieties. Also, the pectin content was nearly the same for all varieties and types. The average of all whole pectin values is 670 μg/ml (670 ppm) which is approximately 25% higher than the RSK max of 500 ppm (Flussiges Obst, 1987). Values obtained for water-soluble alcohol-precipitated pectins which are higher than the RSK limit do not necessarily indicate adulteration of orange juice. As previously reported, differences exist in values obtained for pectin content in orange juice and may be related to several variables, including index of maturity, variety, method of juice extraction, and pulp content (Royo-Iranzo *et al.*, 1977).

Uronic reducing groups generally increased in the whole juice for each variety, with the exception of F125 which remained relatively constant during one week at 5°C storage, indicating the liberation of galacturonic acid as the result of enzymatic activity. Under the relatively high acid conditions of the juice, extracted from clean oranges held cold at 5°C, we believe that significant mold growth was unlikely, therefore the possibility of pectinase enzyme production from mould in this juice was also unlikely. It is also unlikely that chemical degradation of the pectin would occur under

Table 1. Characteristics of freshly squeezed juice and juice stored for one week at 5°C

Variety/Date	%TA	°Brix	I of M	S/P	S/RG	S/DP	W/P	W/RG	W/DP
F113/10–92 in	0·74	12·2	16·5	—	—	—	3·36	0·075	45
F113/10–92 1 wk	0·76	12·3	16·2	—	—	—	2·84	0·086	33
V138/11–92 in	0·74	13·9	18·8	0·67	0·064	10	3·46	0·074	47
V138/11–92 1 wk	0·75	13·5	18·0	0·34	0·048	7	3·57	0·117	31
F150/12–92 in	0·525	9·15	17·4	0·73	0·045	16	2·97	0·085	35
F150/12–92 1 wk	0·53	9·2	17·4	1·25	0·081	15	3·11	0·127	24
F125/1–93 in	0·865	11·5	13·3	0·81	0·046	18	3·02	0·090	34
F125/1–93 1 wk	0·86	11·5	13·4	0·34	0·027	13	2·77	0·087	32

Pectin and reducing group values are in units of μmole/g orange juice.

S = Serum
W = Whole juice
P = Pectin
DP = Degree of polymerization (P/RG)
RG = Reducing groups
TA = Titratable acids (based on citric)
I of M = Index of Maturity (°Brix/%TA)
in = initial time, freshly squeezed
1 wk = 1 week at 5°C storage
F = Florida packing-house source
V = California Valencia

the conditions of the study, based on observations with model pectin-containing systems under similar conditions to this experiment in the absence of enzymes (Doesburg, 1965; Sajjaanantakul, 1989).

The DP decreased by an average of 30% with each variety over a period of one week at 5°C as the whole juice clarified, with the exception of F125 which remained approximately constant. The results for F125 may be reasonable given the relatively low index of maturity designating an immature fruit.

Clarification of orange juice can be prevented by heat treatment, and evidence as previously published suggests that for stabilization of cloud by heat treatment, pectic enzymes other than PE may also play a role (Joslyn & Pilnik, 1961). The polygalacturonases catalyze the hydrolytic cleavage of the *O*-glycosyl bond of α-D-(1→4) polygalacturonan, proceeding either randomly or terminally (*endo* or *exo*), resulting in the release of galacturonic acid. Optimal substrates for polygalacturonases are deesterified pectic polymers or polygalacturonans (Burns, 1991). Fresh orange juice contains active pectinesterase which saponifies the pectin (Krop, 1974), making it a suitable substrate for PG attack.

The initial serum pectin content was nearly the same for all varieties. However, after storage at 5°C, the serum pectin content generally decreased with the exception of F150. Active PG could have depolymerized the pectin, yielding products too small to precipitate with ethanol; hence the values decrease. F150 had the highest average value of DP after one week and was the only case where the serum pectin content did not decrease. Rouse *et al.* (1957) concluded that depolymerization in addition to deesterification may be the cause of differences seen in pectin concentration of juices of similar cloud values. Other data involving the extractability of various pectin fractions collected by Zuegg (1955) imply depolymerization as well as deesterification of the pectic material of orange juice.

The serum reducing group profile behaved similarly to the serum pectin content profile.

In each variety the serum DP decreased, indicating enzymatic depolymerization of the pectin. The decrease varied from less than 10% to 50%. The DP of serum pectin was lower than the overall DP of whole orange-juice pectin.

CONCLUSIONS

The average degree of polymerization of the pectin present in orange juice can be determined after measuring the galacturonic acid and reducing group content of the 80% alcohol-insoluble/water-soluble fraction. The degree of polymerization generally decreased with storage at 5°C, indicating enzymatic degradation of the pectin. It appears that PG is active in orange juice. Its activity is low; therefore clarification occurs due to the continued presence of large pectin chains. These polymers have decreased in size but remain too large to stabilize the cloud.

Further studies relating turbidity of orange juice to precise limits of degree of polymerization of the pectin should be conducted to determine the threshold of cloud stability.

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